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Disease and Diagnosis: The Clinical Laboratory

Early in this book I stated that one of the areas in which cytometry has had great impact is in clinical diagnosis. Research work, beginning with Kametsky's foray into cytological screening, but particularly during the last 20 years, has made it clear that the information that can be obtained by flow cytometry could be of clear use to clinicians. However, as long as flow cytometers maintained their image of cumbersome and fiddly instruments, difficult to standardize and requiring constant attention from a team of unconventional but devoted hackers (see Figs. 1.3 and 1.4 for an understanding of the origins of this image), there was little chance that flow cytometry would become integrated into routine hospital laboratories.

The direct cause of the rapid introduction of flow technology into many hospitals was the commercial development and marketing of "black box" or "benchtop" flow cytometers (in imitation of the pattern set by blood counters, which were, by the 1980s, standard equipment in hematology laboratories). Installation of these benchtop flow instruments involves placing them on a laboratory bench and plugging them in. They are optically stable and therefore can, in principle, be maintained with relatively little human intervention; they can produce clinical printouts that can be read without any requirement for knowledge of the intricacies of flow data analysis; and they can, we are told, be run by anyone with the ability to push a key on a computer keyboard (Fig. 10.1). It was only with the development and marketing of these friendly machines (starting in the mid to late 1980s) that provision of flow cytometric information for routine diagnosis became a practical proposition.

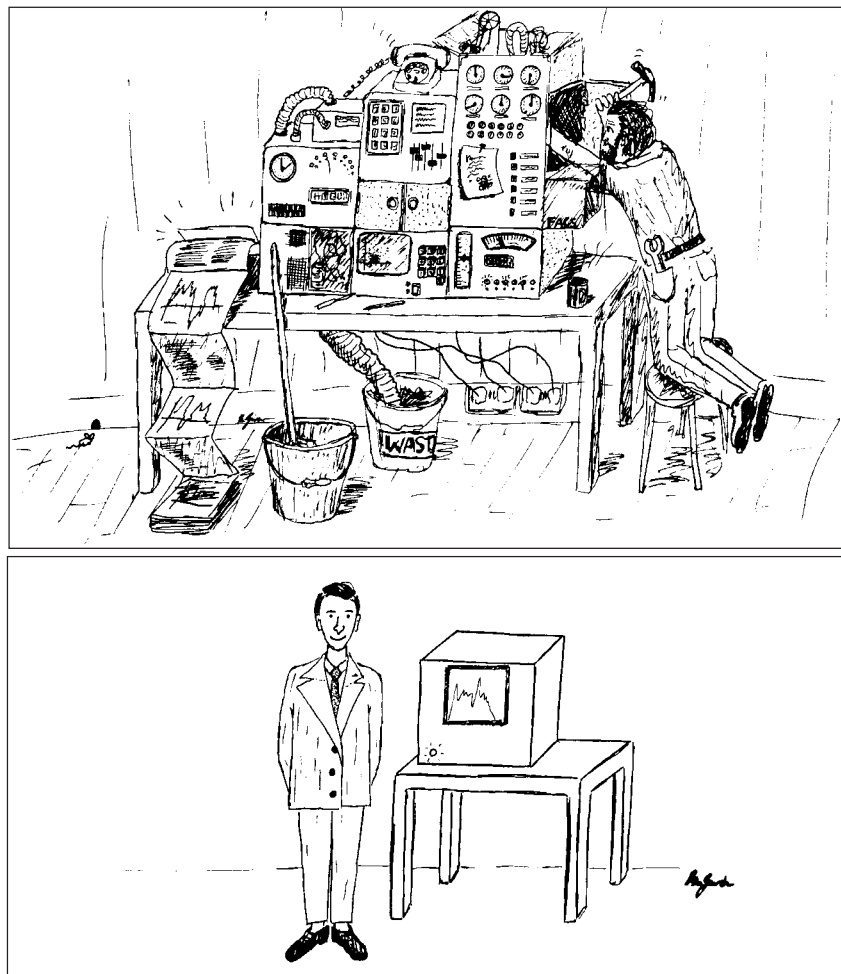


Fig. 10.1. Two opposing fantasies of what flow cytometry is all about. Drawings by Ben Givan.

As a result of the introduction of cytometers into the hospital setting, three aspects of clinical practice have led to some general reassessment of the nature of flow analysis. First, clinical laboratories are, because of the import of their results, overwhelmingly concerned with so-called quality control. This concern has forced all cytometrists to become more aware of the standardization and calibration

of their instruments. Neither standardization nor calibration comes naturally to a flow cytometer. In response to this clinical requirement, beads, fixed cells, and mock cells have been developed to help in assessing the stability of conditions from day to day. Instruments can be set up from stored computer information so that machine parameters are constant from run to run. Furthermore, the analysis of data can be automated so that clinical information can be derived and reported quickly and in standard format. In addition, quality control schemes of various sorts have resulted in samples flying around the world; reports appear in the literature documenting the factors that lead to variation in results obtained from different laboratories and with different operators. Quality control in flow cytometry is still far from secure, but progress is being made toward producing technical recommendations (both for DNA and for leukocyte surface marker analysis), toward providing schemes for accrediting personnel and laboratories, and toward monitoring the performance of laboratories—with comparisons between laboratories and within any one laboratory over the course of time.

The second aspect of clinical practice that has led to a reassessment of the nature of flow cytometry is the occasional clinical requirement for “rare-event analysis.” Methods have been developed, particularly with the use of multiparameter gating, to lower background noise in order to provide increased sensitivity for detection of rare cells. In the clinic, this increased sensitivity translates, for example, into earlier diagnosis of relapse in leukemia, more sensitive detection of fetal–maternal hemorrhage, and better ability to screen leukocyte-reduced blood transfusion products for residual white blood cells. Outside the clinic, these methods for rare-event detection have begun to stretch the limits of research applications as well.

A third aspect of clinical practice that has led to modifications in flow technology has been the requirement for safety in the handling of potentially infectious specimens. The fluidic specifications of instruments have been modified with attention to the control of aerosols that might occur around the stream, the prevention of leaks around the sample manifold, and the collection of the waste fluid after it has been analyzed. However, a more effective means of minimizing biological hazards has been the development of techniques for killing and fixing specimens in such a way that cells, viruses, and bacteria are no longer viable, but the scatter and fluorescent proper-

ties of the cells of interest are relatively unchanged. Formaldehyde (1.0%) is the fixative of choice in most flow laboratories. It is used after cells have been stained. As well as reducing the infectivity of the hepatitis B and AIDS (HIV) viruses, it fixes leukocytes in such a way that their scatter characteristics are virtually unaffected. Moreover, the fluorescence intensity of their surface stain remains essentially stable (albeit with slightly raised control autofluorescence) for several days or more. Therefore, routine fixation of biological specimens has not only increased the safety of flow procedures but has also made it possible to ship specimens around the world and to store specimens within a laboratory for convenient structuring of access to the cytometer (a euphemism for not working in the middle of the night). It should be said, however, that anyone working with material of known biological hazard needs to check fixation procedures to confirm the reduction of infectivity. Even after fixation, anyone working with any biological material at all should use standard precautions for control because any particular fixation procedure might not be effective against unknown or undocumented hazards. Furthermore, whatever fixation procedure is used should be checked with individual cell preparations and staining protocols to confirm the stability of cytometric parameters over the required period of time.

THE HEMATOLOGY LABORATORY

The flow cytometer has, for several reasons, found a very natural home in the hospital hematology laboratory. In the first place, because of the virtually universal use of automated instrumentation for enumerating erythrocytes and leukocytes, people working in hematology laboratories are quite relaxed about the idea of cells flowing in one end of an instrument and numbers coming out the other end. As I have said, both historically and technologically there is a close relationship between flow cytometers and automated hematology counters. The second reason is the obvious fact that blood exists as a suspension of individual cells—so that red and white cells do not need to be disaggregated before flow analysis. The third reason for the hematologist's ease with flow technology is that hematology/immunology laboratories were among the first to make routine use

of fluorescently tagged monoclonal antibodies. For many years, microscopists have been using panels of monoclonal antibodies for identifying various subpopulations of lymphocytes that are suggestive or diagnostic of various disease conditions. In particular, because the leukemias and lymphomas represent a group of diseases that involve the uncontrolled clonal proliferation of particular groups of leukocytes, various forms of these malignancies can be identified and classified according to the phenotype of the increased number of white cells found in biopsy material or in the patient's peripheral circulation.

Leukemia/Lymphoma Phenotyping

Studies of the staining of surface proteins (CD antigens) to determine the phenotypes of the abnormal cells in patients with various leukemias or lymphomas have been useful, revealing much about ways to classify the diseases but also increasing our knowledge of normal immune cell development. In general, immune cells gain and lose various surface proteins in the course of their normal development in the bone marrow until they become the cells (with mature phenotype and function) that are released from the marrow into the peripheral circulation (Fig. 10.2). Leukemias and lymphomas often involve a block in this development so that cells with immature phenotypes appear in great numbers in the periphery. Alternatively, certain forms of disease may involve rapid expansion of a clone of mature normal cells so that one type of cell predominates in the circulation over all other normal subpopulations. In other cases, cells seem to express abnormal combinations of CD antigens, a condition referred to as *lineage infidelity* or as *lineage promiscuity* depending on one's interpretation of the data. These variations in surface protein expression make classification of leukemias and lymphomas a suitable application for flow cytometry, albeit a very complex task. Correlation between classification and predicted clinical course is therefore correspondingly difficult.

Once the phenotype of a blood cell malignancy in a particular patient is known, the cytometrist can use the multiparameter flow description of that phenotype to define the malignant clone and to look for the absence of these cells in order to diagnose remission after

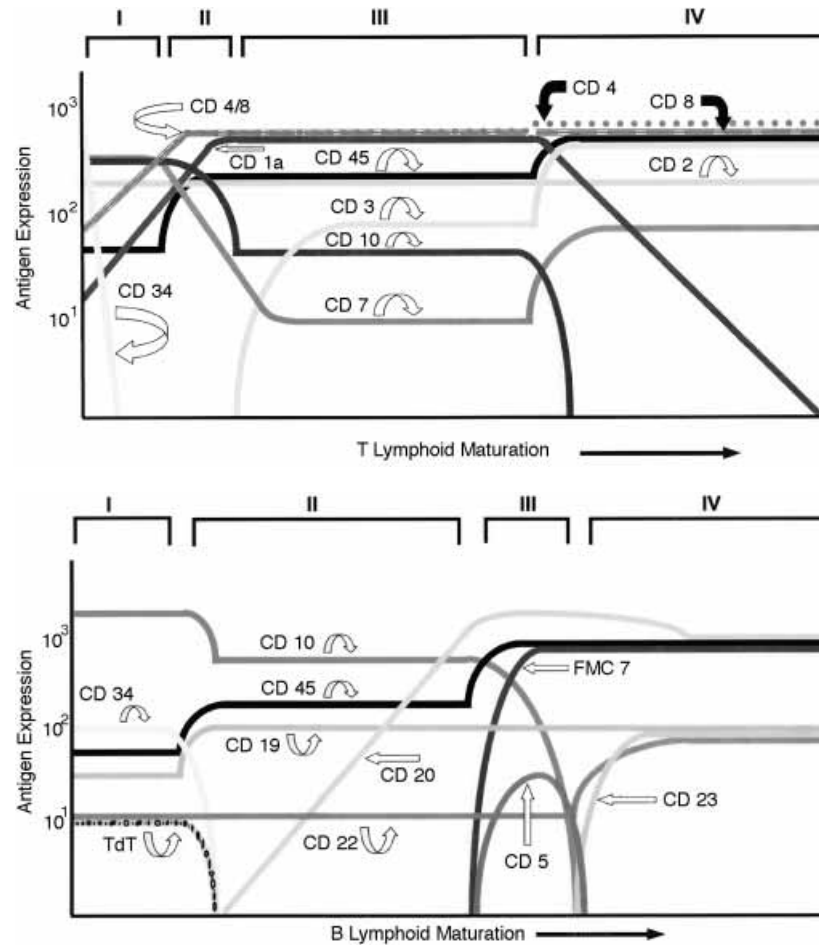


Fig. 10.2. Surface antigen changes during hematopoiesis. The upper plot is of T-lymphocyte maturation and the lower plot of B cells. From Loken and Wells (2000).

treatment. In addition, the flow phenotype of the malignancy can be used to look for the emergence of small numbers of these cells should relapse occur. This, of course, assumes that the relapse phenotype is identical to the abnormal clone defined at the initial diagnosis (Fig. 10.3).

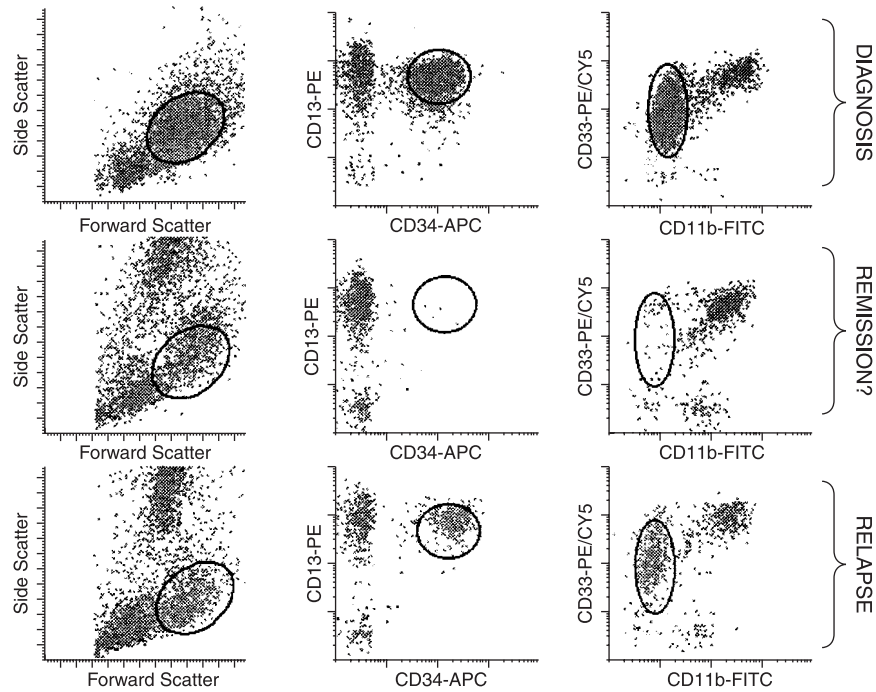


Fig. 10.3. Clusters of cells indicating an abnormal (acute myeloid leukemia) leukemic population, at diagnosis, at remission, and after relapse. The leukemic phenotype is CD13, CD34, and CD33 positive and CD11b negative. Courtesy of Carleton Stewart.

HIV/AIDS

Another condition that involves analysis of peripheral blood leukocytes is AIDS. Early in the natural history of the disease (or at least in the natural history of immunologists' awareness of the disease), it was discovered that one subpopulation of T lymphocytes in particular was destroyed by the HIV virus; the cells destroyed are those that possess the CD4 protein on their surface. It is this CD4 protein that appears to be a receptor involved in virus targeting. Therefore, much of the diagnosis and staging of AIDS involves the enumeration of CD4-positive cells in the peripheral blood (Fig. 10.4).

These techniques—for counting CD4-positive cells in connection with AIDS diagnosis and for phenotyping various populations of

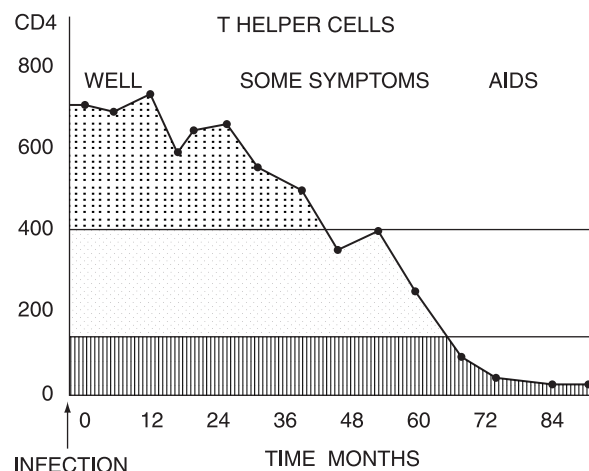


Fig. 10.4. The number of T-helper (CD4-positive) lymphocytes ($\times 10^3/\text{cm}^3$) in peripheral blood of a patient after infection with HIV. Cells <400 are significantly below the normal range; <100 indicates severe risk of clinical AIDS. Courtesy of Léonie Walker.

leukocytes for leukemia/lymphoma diagnosis—can be performed in hematology laboratories by the staining of cells with fluorochrome-conjugated monoclonal antibodies followed by the visual identification of different types of white cells and the counting of the fluorescent versus unstained cells under the microscope. Although the microscope has certain very definite advantages over the flow cytometer, two advantages it does not have are those of speed and statistical reliability. Particularly as a result of their work load from the growing number of AIDS patients, hematologists' need for a way to count statistically reliable numbers of cells from large numbers of patients became increasingly urgent. Flow cytometry was the obvious answer to this need.

Erythrocytes and Platelets

Although the initial perceived need in the hematology laboratory for a flow cytometer was to aid in the rapid processing of samples from leukemic and HIV-positive patients, the presence of the cytometer has

stimulated thought about new hematological applications. Although not yet in routine use for analyzing erythrocytes, flow cytometers have been shown to be useful for looking at red-cell-bound immunoglobulin (as a result of autoimmune disease, sickle cell anemia, and thalassemia): The bound immunoglobulin on erythrocytes is detected by the use of fluorescent antibodies against human immunoglobulin. The staining of red cells for RNA content with a dye called *thiazole orange* has made possible the use of flow cytometry to count the reticulocytes (immature erythrocytes) present in blood samples from anemic patients.

Hematologists have also extended the use of flow analysis to platelets—those particles with low forward scatter that usually are ignored in flow cytometric applications because they fall well below the standard forward scatter threshold. Immunoglobulin bound to platelets can be measured with antibodies against human immunoglobulin (as in the detection of immunoglobulin on erythrocytes). Platelet-associated immunoglobulin (resulting from autoimmune disease) is determined by analyzing the patient's platelets in their natural state. In another clinical situation, antibodies in the serum with specificities for "foreign" platelets may develop after pregnancy or transfusions; these can be monitored by flow cytometry if a patient's serum is incubated with a donor's platelets. Recently, the activation state of platelets has also been analyzed. Hyperactive platelets express P-selectin (CD62) on their surface; they have been primed to facilitate coagulation. Although not yet applied in routine diagnosis, research indicates that expression of CD62 may provide prognostic information in myocardial infarction and cardiopulmonary bypass surgery.

HLA-B27 Typing

Human cells express a series of proteins on their surface that are involved in self-recognition and in antigen presentation to effector cells for the stimulation of immune reactions. These are called *major histocompatibility* (MHC) antigens, and each occurs in a variety of allelic forms. People, therefore, differ from each other in their MHC genotypes. As well as affecting transplant compatibility, certain genotypes have been implicated in predisposition to autoimmune disease.

In particular, HLA-B27 (the 27th haplotype at the B locus of the human leucocyte antigens), has been associated with arthritic conditions (ankylosing spondylitis, juvenile rheumatoid arthritis, and Reiter's syndrome). While only about 20% of people with the HLA-B27 genotype have ankylosing spondylitis, for example, a very high percentage (perhaps 90%) of people with this disease are HLA-B27 positive (compared with 4–8% in the general population). Antibodies against the HLA-B27 protein are available. Leukocytes can be incubated with fluorochrome-conjugated antibodies; positive staining indicates expression of the HLA-B27 protein. This assay can be performed with a microscope (using either fluorescent antibodies or complement-fixing antibodies that kill cells). Recently, increasing numbers of hematology laboratories have begun to use flow cytometers for this assay.

CD34 Stem Cells

After a cancer patient has been treated with irradiation to destroy malignant cells, normal blood cells will also be depleted and the patient may require transplantation to regenerate these cells. Hematopoietic stem cells (classified by possession of the CD34 antigen on their surface) are immature, undifferentiated cells that have the ability to differentiate into all classes of blood cells. They exist, normally, in the bone marrow; detectable numbers can be found in the peripheral blood if the patient has been treated with blood cell growth factors. If enough of these stem cells can be harvested from the patient before irradiation, transplantation of these cells back into the patient after irradiation can replenish the immune system. The number of these CD34-positive cells given back after irradiation needs, therefore, to be assayed in order to ensure that the stem cell transplant is sufficient to return the patient to normal immune competency. Because these stem cells are uniquely characterized by the CD34 antigen, flow cytometry can be used to confirm the presence of sufficient stem cells in a cell suspension obtained from the patient before irradiation (Fig. 10.5). Because the actual number of these stem cells (rather than just their proportion in a 10,000 cell data file) is important, the volume flowing through the flow cytometer can be calibrated by the addition of known numbers of beads to the sample.

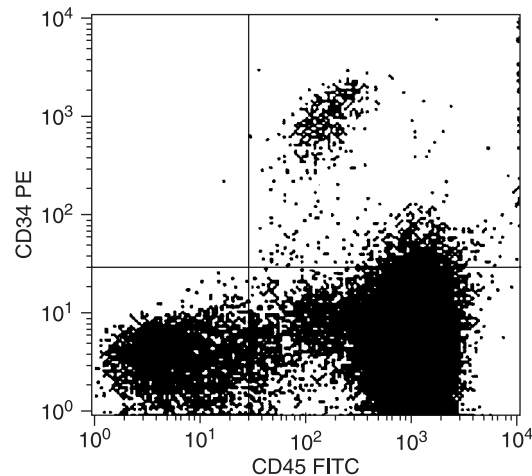


Fig. 10.5. Stem cells can be assayed by the use of antibodies against the CD34 and CD45 antigens. From R Sutherland as published in Gee and Lamb (2000).

Fetal Hemoglobin

Detection of fetal erythrocytes in the maternal circulation is important in diagnosing a cross-placental fetal–maternal hemorrhage that might have resulted from trauma with suspected maternal injury. In addition, in the case of Rh-blood group incompatibility between fetus and mother, rapid detection of fetal cells in the maternal circulation can allow early intervention and the prevention of Rh-incompatibility disease (erythroblastosis fetalis) in the newborn. Although detection of these fetal cells is difficult because their proportion in the maternal circulation is low (less than 0.1% in a normal pregnancy; perhaps 0.6% in a pregnancy that needs therapeutic intervention), they can be assayed by the use of antibodies against the fetal form of hemoglobin (HbF). This procedure is typical of flow protocols concerned with rare-event analysis (Fig. 10.6). Defining the cells of interest (in this case the HbF-positive cells) by multiple parameters (forward scatter, side scatter, autofluorescence, HbF) reduces the likelihood of false positivity resulting from background noise.

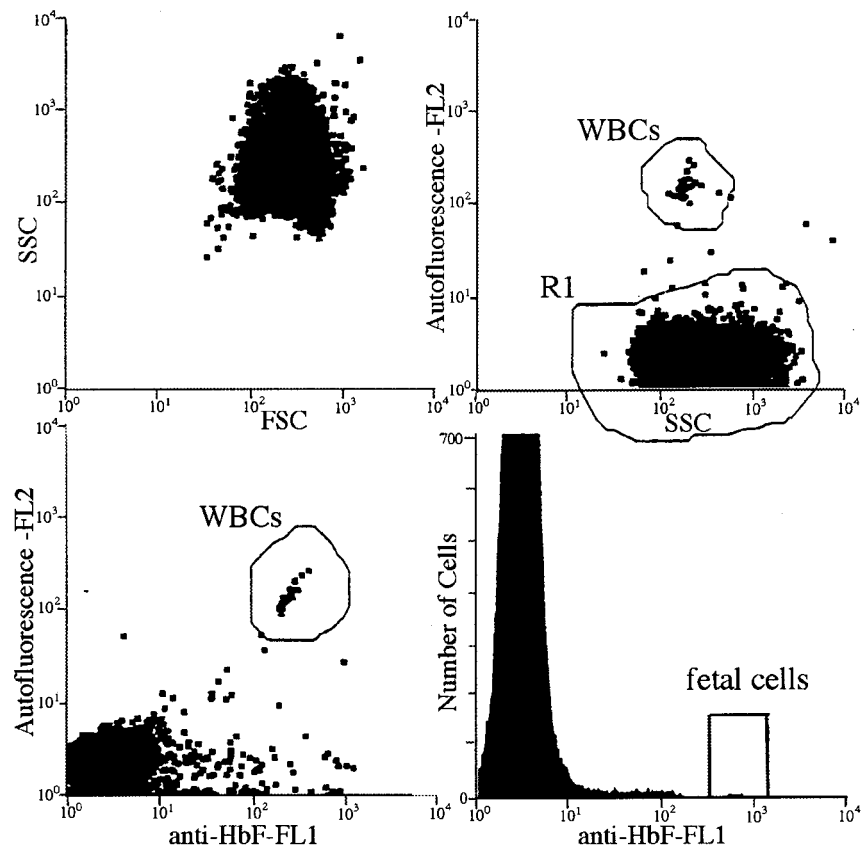


Fig. 10.6. Detection of rare fetal erythrocytes in the maternal circulation. The fetal erythrocytes are HbF positive and low in autofluorescence. The cells in R1 are displayed and enumerated in the bottom right histogram. From Davis (1998).

THE PATHOLOGY LABORATORY

Tumor Ploidy

In the chapter about DNA analysis, I mentioned the large amount of work generated for flow laboratories as a result of publication of the Hedley technique for analyzing the DNA content of paraffin-embedded pathology specimens. After the first headlong rush of publications correlating DNA ploidy with long-term prognosis in

various types of cancer, the field has now settled down a bit. As I have indicated, it would probably be fair to say that most (but not all) studies on disaggregated solid tumors (fresh, frozen, or fixed) have shown some kind of correlation between abnormal DNA content and unfavorable long-term prognosis.

There is considerable debate in the literature about whether flow cytometric analysis of ploidy gives any additional prognostic information that is independent of other known prognostic indicators, but most studies have indicated that it does. It appears to be useful, for example, in indicating, from all breast cancer patients without lymph node involvement, a group of women who are at risk for recurrence of disease (Fig. 10.7). Because any pathological material will be a mixture of both normal and abnormal cells, there is much current effort expended on ways of selecting from among the cells of a disaggregated specimen those particular cells that are from the tumor itself. If those tumor cells can be stained selectively (as with a fluorescein-conjugated anti-cytokeratin antibody for epithelial cell-derived tumors within nonepithelial tissue) and then the DNA con-

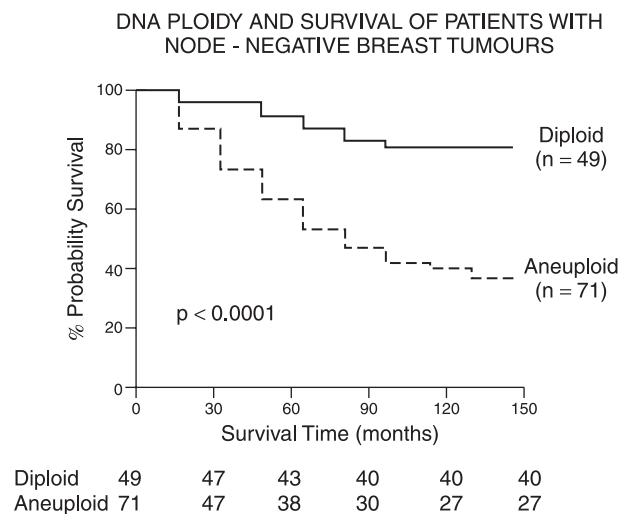


Fig. 10.7. Survival curves for breast cancer patients without lymph node involvement. Those with DNA aneuploid tumors (as diagnosed by propidium iodide flow cytometry of 10-year-old paraffin-embedded material) survived significantly less long than those with DNA diploid tumors. Graph from Yuan et al. (1991).

tent of the fluorescein-stained cells determined, we may have a dual color flow method of much improved sensitivity for detecting aneuploid cells from within a mixture of both malignant and normal components.

S-Phase Fraction

There is also evidence, in solid tumors as well as in the bone marrow plasma cells from patients with multiple myeloma, that determination of the proliferative potential of malignant cells is a better indicator of poor prognosis than simply the determination of the presence or absence of aneuploid cells. As discussed in the chapter on DNA, proliferation (the proportion of cells in S phase) can be assessed by using mathematical algorithms to analyze the shape of the DNA histogram. However, the presence of more than one cell type, with the G2/M peak from the diploid cell line overlapping the S phase of the aneuploid cells, makes mathematical analysis even more complex than with distributions resulting from single euploid cells or clones (Fig. 10.8). Nevertheless, correlations between high S-phase fraction and poor clinical prognosis appear relatively strong. Bromodeoxyuridine (BrdU) has been used in the research setting to give information about proliferation; it has been used both in vitro with fresh excised tumors and in vivo by infusion into patients at some time before

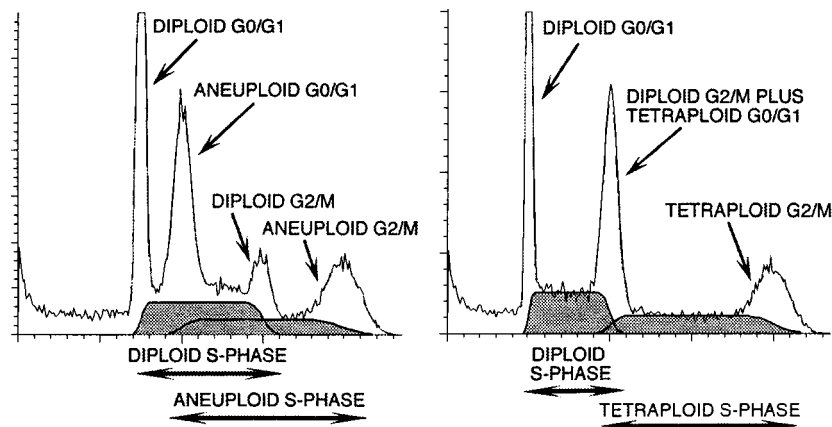


Fig. 10.8. Analysis of mixed diploid/aneuploid cells for the S-phase fraction of the aneuploid population.

excision of the tumor. Estimates of tumor doubling time, based on the rate of movement of BrdU-pulsed cells through the cell cycle and on the percentage of cells in the tumor that are dividing, have been shown to correlate with aggressiveness of the malignancy. There may in the future be more use of this technique for assaying malignancies for sensitivity to various cytotoxic drugs.

Ploidy and S-phase fraction determinations are, in most cases, the only flow analyses that have achieved much routine application in the field of solid tissue oncology. At the present time, it would appear that flow cytometry has not settled quite so comfortably into the pathology laboratory as it has into the hematology setting. This may, I suspect, come from the crucial difference between hematology and pathology—and it is a difference that may remind us of one very definite limitation of flow analysis. Pathological specimens come from solid tissue; to analyze them with a flow cytometer, the solid tissue must be disaggregated in some way. This disaggregation process (either mechanical, with detergent, or with enzymes) not only has a good chance of compromising the integrity of some or many of the cells we want to analyze, but flow cytometry will also, necessarily, lose any information that is contained in the structural orientation or pattern of the original cells and tissue, as viewed under the microscope.

A response to this dilemma has come, recently, in the development of laser-scanning cytometry (the LSC by CompuCyte). The LSC uses a laser to scan cells or tissues on slides and acquires data that can be presented much as any flow cytometric fluorescence or light scatter information (in histograms or two-color plots). However, the LSC also acquires extra parameters that record the x/y position of each cell on the slide. In this way, cells that show aberrant fluorescence or scatter characteristics can be examined individually under the LSC's microscope to provide high-resolution images and confirm malignant or otherwise interesting phenotype. In many ways, the LSC bridges the technologies of flow cytometry and traditional microscopic pathology.

SOLID ORGAN TRANSPLANTATION

It has been known for many years that transplant recipients may possess serum antibodies that will react with and destroy cells from a transplanted organ. If these so-called cytotoxic antibodies are present at the time of transplantation, then an immediate and violent rejec-

tion crisis will occur (hyperacute rejection), destroying the grafted organ and threatening the life of the recipient. Therefore, the serum from a potential recipient is assayed before surgery for cytotoxic antibodies by mixing it with lymphocytes from the organ donor (in the presence of rabbit complement). Traditionally, the cytotoxicity of the serum in this cross-match assay is scored by counting dead lymphocytes (cells taking up propidium iodide or trypan blue) under the microscope. Addition of anti-human globulin can enhance the sensitivity of this assay for bound immunoglobulin.

In the early 1980s, MR Garovoy and his group in California suggested that this cross-match assay might not be sensitive enough. Some of the cases of slower rejection might result from the presence in the organ recipient of levels of preformed antibodies that were too low to be detected by the traditional cytotoxic assay. A flow cytometric cross-match assay for these preformed antibodies directed against donor lymphocytes was developed by Garovoy and subsequently modified by Talbot in Newcastle and by workers at other centers to make use of two-color fluorescence. The assay involves the incubation of the organ donor's lymphocytes with serum from potential recipients followed by the staining of the lymphocytes with a phycoerythrin-conjugated monoclonal antibody against either T cells or B cells and a fluorescein-conjugated antibody against human immunoglobulin (Fig. 10.9). In that way, it can be determined whether the recipient has antibodies that coat the T lymphocytes or the B lymphocytes of the donor (Fig. 10.10). This two-color technique (or three-color, if T- and B-cell antibodies are used simultaneously) can in fact serve to remind us of a general approach to the design of flow cytometric assays: One color is used to pinpoint the cells of interest (in this case, either T cells or B cells), and the second color is used to ask some question about those cells (in the cross-match, we are asking whether those T or B cells have become coated with immunoglobulin from the recipient's serum).

Evidence over the past 15 years has indicated that this assay for lymphocytes with bound antibody is indeed more sensitive in detecting antibodies directed against donor cells than is the traditional (microscope) assay for lymphocyte death. The flow assay also defines a group of recipient-donor pairs who are at risk for severe rejection crises and possibly, but not necessarily, for loss of the grafted organ. At the present time, donated organs are in short supply. One transplant

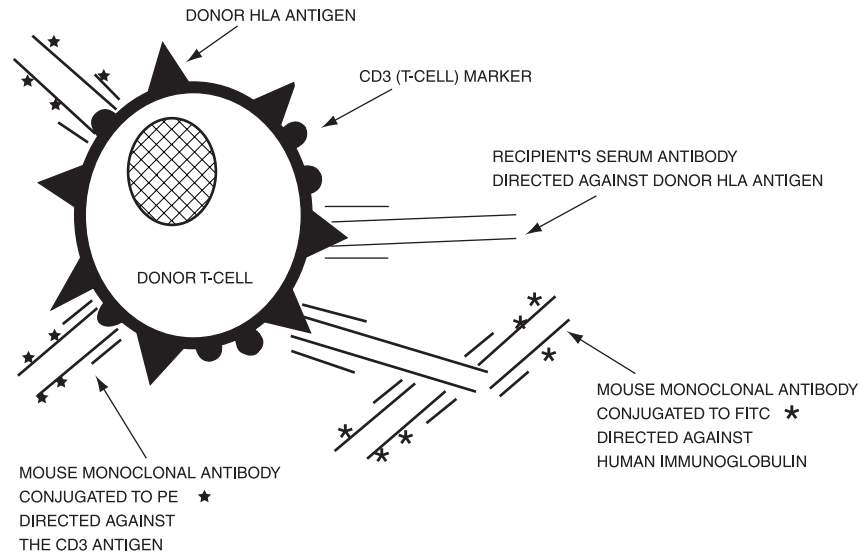


Fig. 10.9. Protocol for the dual-color flow cytometric assay for testing recipient serum for the presence of antibodies to donor cells before organ transplantation.

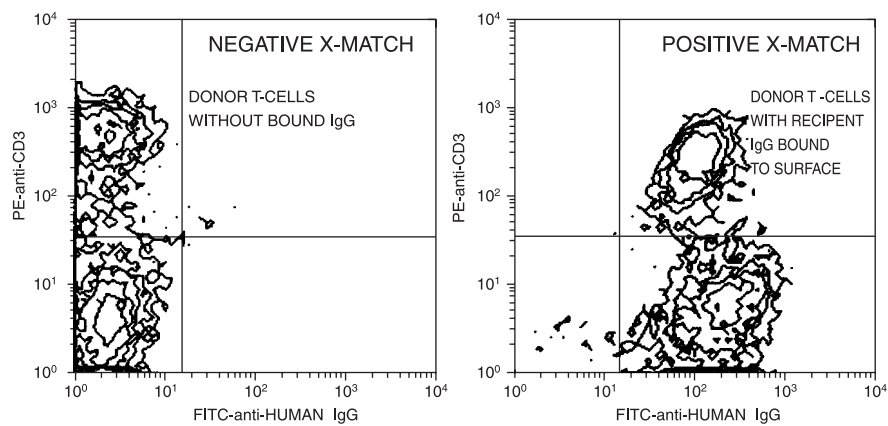


Fig. 10.10. Contour plots indicating a cross-match assay for immunoglobulin from the recipient bound to the surface of the donor's T cells. Data from the Tissue-Typing Laboratory, Royal Victoria Infirmary, Newcastle upon Tyne.

surgeon has stated persuasively that, in selecting patients for transplantation, he is most concerned with making sure that the donated kidney will survive. Although the flow cytometric cross-match assay may arguably be denying some recipients unnecessarily the opportunity of a successful transplant because it may be too sensitive, it certainly is helping surgeons to find a group of patients who have the very best chance of accepting the transplanted organ. Because transplant surgeons have few organs to graft and long waiting lists of potential recipients and because not enough is yet known about ways to predict, diagnose, and abrogate rejection, the use of flow cytometry has been accepted relatively quickly into routine use by some but not all of the transplant community.

The acceptance of the flow cross-match into the hospital environment highlights some of the difficulties that occur when any technique moves from research into service use; in the case of transplantation surgery, some of these difficulties are particularly acute. The flow cross-match needs to be implemented in such a way that it can be performed by people who may be working in the middle of the night under pressure of time and without specialist support staff available for easy consultation. Under such conditions, automated software and stable instrumentation are especially important. In addition, flow cytometers are relatively expensive instruments; tissue-typing laboratories are concerned with ways to justify the expense of a cytometer because it needs to be available all the time, but it may only be used on infrequent occasions. However, the most serious problem in implementing the flow cross-match for routine use has been in defining the position of the dividing line between negative and positive results. With increasing instrument sensitivity, large numbers of patients will show some level of serum antibodies directed against donor cells. Definition of a fluorescence intensity borderline for proceeding with or denying an organ transplant clearly has to involve some understanding of the clinical (and ethical) requirements of patients and of the supply and demand for organs as well as of the technical capabilities of the cytometer.

COMMENTS

Rational decisions still need to be made about the fields in which flow cytometry can make a positive contribution to patient care, about the

methods available for quality control, and about the kind and depth of training required by staff. Good channels of communication also need to be opened between laboratory staff and the clinicians who are making diagnostic use of flow data, but who may not be aware of its limitations and conventions. Benchtop cytometers are optically stable and can be used and maintained with remarkably little human intervention. Software packages for those cytometers can apply algorithms for automated lymphocyte gating of blood preparations—the computer will draw a lymphocyte gate for you. The future may contain more sophisticated expert systems employing artificial intelligence in order to avoid the pitfalls associated with human judgement. How stable these instruments are in practice, whether automated computer algorithms make reasonable guesses with difficult samples, and whether an untrained scientist or clinician can draw reliable conclusions from automated print-outs of flow data are open questions. With flow cytometry, as in any field of science, we need to guard against conveying a false sense of objectivity to laymen not familiar with the subjectivity of a technique.

Kenneth Ault, the former president of the International Society for Analytical Cytology, summed up some of flow cytometry's clinical growing pains with the following statement:

Flow cytometry is a technology that seems to stand at the threshold of "clinical relevance." Those of us who have been using this technology, and especially those who are manufacturing and selling the instruments and reagents, are frequently evaluating the status of clinical flow cytometry. Most of us have little doubt that this is going to be an important technology in clinical medicine for many years to come. However, from my point of view, and I think for many others, the movement into the clinic has been unexpectedly slow and painful. . . . In talking about this in the past I have frequently mentioned the "grey area" between research and clinical practice. I was recently reminded of a quotation from T.S. Eliot: "Between the idea and the reality falls the shadow." I believe that flow cytometry is currently traversing that shadow. KA Ault (1988). *Cytometry* (Suppl 3):2–6.

That paragraph was written for a talk given in 1986. In the first edition of this book (1992), I said that clinical cytometry was, at that time, in what we might call dappled sunlight. Fourteen years after Ault's talk, in 2000, Bruce Davis, the president of the Clinical Cytometry Society (Newsletter 5:1–2) said that he remains

cautiously optimistic regarding the future of diagnostic cytometry in areas of both image and flow cytometry. Although the applications of lymphocyte subset counting have seemed to reach a plateau, new applications using cytometry techniques for disease diagnosis, monitoring, and prognostication continue to appear.

Flow cytometry appears fairly comfortable, now, in many hospital laboratories, and most cytometrists might agree with Davis's cautious optimism. It may be, however, that flow cytometry has not yet reached the full glare of total integration into the hospital community.

FURTHER READING

There are many books that cover, in detail, the aspects of clinical flow cytometry that I have mentioned in this chapter and many other aspects as well. Some of the books that I have found most useful are listed here.

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